Integrated Water Quality and Aquatic Communities Protocol – Wadeable Streams

Standard Operating Procedure (SOP) #22: Data Analysis and Reporting

Draft Version 1.0

Revision History Log:

Previous Version	Revision Date	Author	Changes Made	Reason for Change	New Version

Overview

This SOP describes the general philosophy and approach for data analysis and reporting of the Klamath Network Wadeable Streams protocol. The reports are intended to meet the needs for consistent and appropriate water quality condition reporting and for rigorous, quantitative descriptions of the physical, chemical, and biological aspect of park streams and their changes over time. This SOP is separated into two sections: (1) General intent, philosophical, target audiences, and recommended analysis approaches for Annual Reports, Analysis and Synthesis Reports, and Resource Briefs; and (2) Specific guidelines for water quality and aquatic community analyses. The purpose of section one is to dictate the reporting schedule and content of the reports so that they meet protocol objectives.

These reports are intended to be authored by the Project Lead with assistance from the Network Coordinator and Data Manager and with potential input from outside scientists or science communication professionals, as appropriate. The purpose of section two is to ensure that statistics used in all reports are properly and consistently done. Since water quality assessments can provide a basis for changes in management that might affect public health or livelihoods, we provide specific guidelines to ensure that they maintain continuity over time and conform to appropriate standards and guidelines.

Reporting

The target audience of reports (Annual and Analysis and Synthesis) is a broad group of interested parties, including park superintendents, resource managers, Inventory and Monitoring staff, external scientists, partners, and the public. Resource briefs will be targeted to park superintendents, interpretative staff, and park managers. The timelines and specific purposes of each report are detailed in Table 1.

Annual Reports

Annual reports serve as the main conduit for informing the audiences of the current years' monitoring activities and for conveying water quality condition information. An example of an annual report is given in Appendix A of this protocol and should serve as a template for future reports. In all annual reports, an emphasis will be put on using summary statistics (measures of central tendency and dispersion) for key water quality and community parameters of the protocol. Findings of special interest to resource managers or the public will also be highlighted. Examples of this are instances of wildlife diseases or new records of non-native species. In general, the annual reports will not lend themselves to hypothesis testing; rather, hypothesis testing (on trends) will be covered in later Analysis and Synthesis reports. However, the nature of water quality monitoring allows for the allocation of impaired versus unimpaired condition. This should be a component of all Wadeable Streams annual reports. Recommendations for protocol revisions will also be suggested as necessary.

Annual reports will follow the formatting guidelines of the Natural Resource Publications series, using the Natural Resource Technical Report (NRTR) guidelines. Since the annual reports will include analyses of impairment, it is recommended that the reports always be published in the NRTR series, not the Natural Resource Data Series.

As part of this series, the Annual Report will include:

1. Executive summary.
2. Introduction with brief explanation on project background.
3. Methods section, referencing this protocol, with a level of detail appropriate for scientific publication.
4. Summary of past Network efforts in relevant park units.
5. Results of the current sampling effort, with special reference to species observed.
6. Evaluation of water quality condition (e.g., impaired vs. unimpaired; fair, good, etc.) based on appropriate EPA and state guidelines, for the following: EPA EMAP vertebrate MMI, EPA EMAP invertebrate MMI, state IBIs, Observed/expected ratio, alkalinity, temperature, pH, total nitrogen, dissolved oxygen, and chloride.
7. Public interest highlights.
8. Suggestions and justifications for any proposed changes to the protocol.

SOP #22: Data Analysis and Reporting (continued).

Table 1. Overview of data reporting for Klamath Network Streams Protocol. Year refers to the year initiated (reports will be due the following year). *Analysis and Synthesis reports in 2024 and beyond do not have a "scheduled" topic. Rather, the Network staff at that time is encouraged to explore new and emerging avenues of summaries and analyses (with emphasis on park relevant material), but will always include a trend component. CDF = Cumulative Distribution Function, IBI = Index of Biotic Integrity, O/E = Observed/Expected ratios.

Report type	Year(s)	Purpose	Method and References (if applicable)
Annual Report	Every sampling year	Summarize monitoring activities	
		Describe current status and condition of water quality parameters	Means/Variance/CDF (Stoddard et al. 2005)
		Document changes/recommendations to monitoring protocols	SOP #23: Revising the Protocol (this document)
		Increase communication between I&M program and all parties	
Analysis and Synthesis	2013	Description of Klamath Network Stream Physical gradients and patterns	Kaufmann et al.1999
	2016	Description of Klamath Network Stream Chemistry	
	2019	Description of Klamath Network Stream Community gradients and patterns	Stoddard et al. 2005
	2022	Integrated Assessment of Klamath Network Riparian Habitats	
	2025	Trend Analyses of Ecological Integrity (Select univariate & multivariate - IBI, O/E, species composition)	Time series (e.g., Mann-Kendall; progressive change) (Chatfield 2004; Phillipi et al. 1998)

SOP #22: Data Analysis and Reporting (continued).

Proportion of Streams Condition

Assignment of proportion of different stream conditions based on water quality standards or bioassessment is a standard method for describing monitoring results (Stoddard et al. 2005, US EPA 2006). Generally, the number of miles (or kilometers) affected is the standard and is calculated by tallying the number of miles represented by the affected site and summing up across all "impaired" miles. Tools to implement this approach are available in the *psurvey.analysis* R library provided by the EMP at: http://www.epa.gov/nheerl/arm/analysispages/software.htm.

For ease of calculation, we take a more conservative estimate based on a simple proportion impacted sites to non-impacted sites. For example, if the North Coast B-IBI (see below) has nine out of 30 sites that are graded as "fair," then the total percent of "fair" streams is 30%. This method can be used for O/E scores, IBIs, and proportion of streams exceeding water quality standards. The best way to graphically present this is Empirical Cumulative Distribution Estimates. An example is shown in Figure 1, from (Stoddard et al. 2005), where the horizontal axis is the response variable and the vertical axis is the cumulative percentage of stream length (we will use percentage of sites). Note that it would be possible to create Empirical Cumulative Distribution Estimates for almost every parameter measured in this protocol, for each park monitored; this could result in a very large number of graphs. Instead of presenting countless graphs in annual reports, this technique should be relegated to helping the Project Lead as an Exploratory Data Analysis and to interpret and understand the data collected.

Confidence intervals of the Cumulative Distribution Estimate can be calculated from (Stoddard et al. 2005):

$$Half - width = Z_{1-\propto} * 100 * \sqrt{\frac{p(1-p)}{n}}$$

Where *Z* is the critical value from the student's *t* distribution, *p* is the proportion, and *n* is the sample size. In the Klamath Network, we will use a conservative α level of 0.10 (i.e., a 90% confidence interval) so that for a sample size of 30, the *Z* value is 1.697 (Rohlf and Sokal 1995). Although *p* varies, it is maximized at *p* = 0.05. Under these parameters, the most conservative half-width of our confidence intervals is 15.5%.

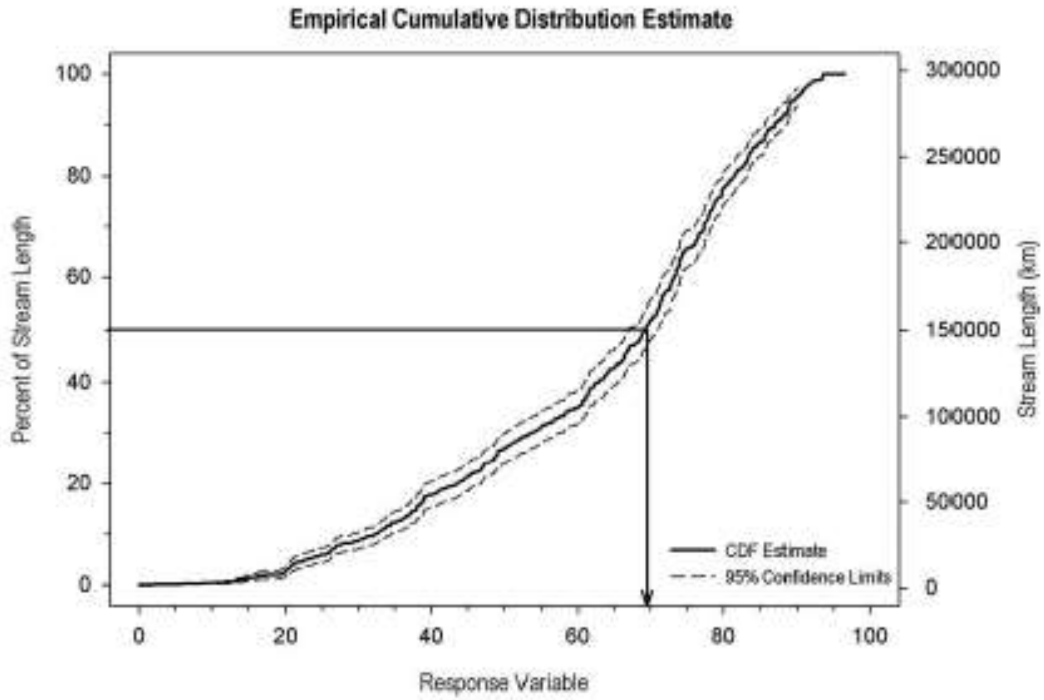

Figure 1. Example of Empirical Cumulative Distribution Estimate for display water quality data. The horizontal line (at 50%) represents the median, which in this example relates to approximately "70" of the response variable.

Analysis and Synthesis Reports

Analysis and Synthesis reports provide in-depth examinations of topics relevant to park management, creating a forum for integrating current science and statistical techniques with Network data. Like the Annual Reports, they should follow the guidelines of the Natural Resource Technical Report, yet they should address identified topics of clear ecological and management significance that have been identified to serve specific audiences within the parks or the larger scientific community. The contents of the report should be similar to scientific publications, with, at a minimum:

1. Abstract
2. Introduction
3. Methods
4. Results
5. Discussion

Depending upon length, complexity, and target audience, Analysis and Synthesis reports may be published in the NRTR series or in peer-reviewed scientific publications.

Analysis and Synthesis Reports 1 – 3: Physical, Chemical, and Biological Gradients and Classification

The reports are planned to be prepared to provide a portfolio of information about our stream ecosystems arrayed as distinct but mutually reinforcing suites of information. They are planned to start with the least temporally variable geomorphic and general hydrological habitat features and move towards the more temporally variable chemical and biological species. We recognize that hydrology (e.g., the hydrograph) itself can be among the most temporally dynamic of stream parameters, but intend to describe the fundamental spatial patterns of the stream networks in each park as a foundation for subsequent temporal analyses of all parameters. An analogy would be to present the general climate features of the parks which are striking and largely consistent over time, even as daily and seasonal weather show great variability.

Analysis and Synthesis Report 1: Stream Physical Habitat Gradients and Classification – will describe the physical habitat gradients and relevant geomorphic types across the sampling frame in each park. It will be prepared after the second sampling period (2013), so that a single season of sampling has occurred in all Network parks. The first report will analyze and synthesize a large set of data, including, but not limited to: geomorphic (e.g., channel cross-section, stream gradient), large woody debris, substrate, discharge, watershed area, and stream order data to provide a comprehensive view of the array and interrelationships of physical stream habitats across the Network parks. Where possible, it will place the park streams within existing geomorphic and stream classification systems (e.g., Strahler 1957, Pfankuch 1975, Frissell et al. 1986, Rosgen 1994, Montgomery and Buffington 1997) to foster comparison with other regional landowners and to provide context for future assessments of ecological integrity in the parks. Flow regime, quantity and size of sediment, and the topographic setting are known to set geomorphic thresholds that define changes in fluvial processes and form, separating riverine landscapes and habitats from one another (Church 2002). This variation in pattern and process comprises important dimensions of the stream ecosystem, with direct relevance to evaluations of ecological integrity (Sullivan et al. 2004). Although geomorphic conditions are transient at a given point along a stream network, the analysis and synthesis of fluvial forms and their interdependence with flow patterns will provide both quantitative descriptions of the stream template and insights into the factors creating and maintaining aquatic and riparian habitat in the parks.

Outside of the variable hydrograph, physical factors of a stream are among the more predictable, compared to the chemical and biological attributes of a stream (Gordon et al. 2004). In addition, the physical template of the stream has a large influence on the abundances, distribution, and productivity of stream organisms. Understanding the available stream types and their attributes provides context for future reports.

Assignment of stream habitats to specific classification units will follow standard rule-based systems (Rosgen 1994) to assign classes. However, such efforts may be aided with multivariate techniques for habitat classification the data collected (NMS, PCA; McCune and Grace 2002). These multivariate techniques will assist in defining classes and understanding the amount and distribution of each stream type identified. Additional incorporation of GIS applications will describe how these streams are distributed across the park landscape.

Analysis and Synthesis Report 2: Water Chemistry Patterns and Correlates addresses a fundamental dimension of water quality that varies in space and time. Stream chemistry is known to vary due to fundamental watershed attributes (watershed geology, stream gradient) as well as transient dynamics of many types, such as vegetation growth, atmospheric dynamics, and pollution (Allan et al. 1997, Scott et al. 2002). This report will analyze and synthesize the spatial patterns in water chemistry to identify major water quality units of functional equivalence (low internal variance) and to identify areas with reference values or potential vulnerability. A goal will be to identify streams with both impaired and outstanding water quality features to inform managers.

Data analyses will aim to identify functional units within the stream reaches sampled in each park through standard multivariate techniques for continuous data (NMS, PCA; McCune and Grace 2002), to identify relationships between watershed parameters (e.g., geology of the basin) and chemical attributes in park landscapes (as in Clow and Sueker 2000), and if appropriate, will include development interpolated models using Geographic Information Systems (GIS) to rapidly convey spatial patterns across the stream networks of the parks. Predictive GIS models may include spatial (purely spatial autocorrelative models, such as kriging) and nonspatial mixed regression models (e.g., General Linear Models), or through models that use both (cokriging; Jager et al. 1990).

Analysis and Synthesis Report 3: Distribution and Abundance of Focal Species and Biological Communities will focus on the biological communities of the network streams (amphibians, macroinvertebrates, and fish).

For each of the biological assemblages of interest, a related set of analyses will be conducted. For species-rich invertebrate assemblages, standard ordination and classification techniques will be used to identify the major units and to portray their correlation with major environmental gradients in each park (Tate and Heiny 1995, Heino et al. 2003). The species diversity, relative abundance, and productivity of assemblages will also be summarized and compared across sample frames and across parks. Factors associated with differences in abundances and distribution will be analyzed using bubbleplots and biplots, using the physical and chemical factors examined in previous Analysis and Synthesis reports. How the physical and chemical templates influences the observed biological patterns will be a major component of this report, so that the parks can understand their biological resources in the proper context.

For fish and amphibian species, which are likely to be considerably poorer in species, analyses will seek delineation of habitats with relatively high abundances of individuals or juveniles, and how the productivity of these vertebrates interact with the physical and habitat template. Both the vertebrates and invertebrates will also include GIS applications to understand their distribution across the landscape. Information about potential stressors (e.g., land use patterns, road distribution, and atmospheric deposition) will also be included in these analyses to gain knowledge about known stressors and biological patterns. The importance of the physical and chemical factors will also be tested using the previous classifications of streams for previous reports for *a priori* groupings, which can be tested using Multi-Response Permutation Procedure or Analysis of Similarity (Clarke and Warwick 2001, McCune and Grace, 2002).

An additional analysis to be included in this report will be matching environmental to biota by maximizing observed correlational strengths using subset of environmental data (Clarke and Warwick 2001). Although an exploratory analysis, this will help identify key environmental variables having the most influence on vertebrate and macroinvertebrate communities.

SOP #22: Data Analysis and Reporting (continued).

In aggregate, the first three Analysis and Synthesis reports will illustrate interrelationships among species assemblages at sites and parks and will be invaluable for distinguishing spatial from temporal variation in subsequent trend detection analyses (Philippi et al. 1998), discussed below under Analysis and Synthesis Report 5.

Analysis and Synthesis Report 4: Instream and Riparian Communities

Riparian zones are focal habitats for many elements of park biodiversity. The fourth report will focus on integrating the instream communities and processes with riparian data collected by this protocol and with data collected by the Klamath Network Vegetation Monitoring and Landbird Community Monitoring Protocols, where possible. The report will explore the co-varying biological communities and their relationships with fundamental landscape gradients in climate, physical form, and water chemistry.

One method of relating these varied assemblage datasets will be with second stage NMS, where rank correlations between similarity matrices are used to display the relationships of the instream communities (fish, amphibians, invertebrates) to the riparian vegetation, landbird communities, and associated environmental variables. Correlative biplots of secondary variables drawn from the riparian and bird data will also be used to examine relationships of these diverse groups to look for similarities. These second stage ordinations will allow the examination of these diverse groups of data collect amongst the three protocols simultaneously (Clarke and Warwick 2001) and also provide a comparability study amongst the protocol. For instance, all three protocols are collecting vegetation data and their results should be broadly comparable (or if not, the *how* they are different will be examined). Knowing and investigating the interrelations between these different vital signs will set the stage for the trends analyses of all three vital signs.

Analysis and Synthesis Report 5: Trend Analyses

Analysis and Synthesis Report 5: Trends in Environmental Conditions and Environmental Integrity will be the first analysis of temporal changes in selected parameters. This will be performed after a total of five sampling periods, so that the sample size for a temporal effect will still be limited. Doing trends analyses before this point, although a major goal of this protocol, would be premature.

The trend report will be analyzed with a variety of parametric and non-parametric techniques, on both univariate and multi-variate parameters (Table 2). In general, in assessing and ascribing change in park ecosystems, a "weight of evidence approach" will be undertaken. In other words, ecosystem changes should be evidenced by multiple, interrelated pieces of physical, chemical, and biological evidence. From such a perspective, redundancy is essential. Changes in species composition over time in a diverse assemblage, therefore, provide more weight of evidence for change than declines in the population of a single species. We also suggest that there are two approaches incorporating “weight of evidence.” The first is that important changes should also be detectable by multiple analytical approaches (following the ideas of R. Irwin, personal communication). For instance, if several tests (Mann-Kendall, regression, *and* multivariate) all agree that a significant change has occurred, this will be taken as strong evidence of biologically significant change, whereas a single test showing significant change (e.g., only the Mann-Kendall) will be taken as weaker evidence of biologically significant change. The second approach is a more traditional, where multiple indicators respond (e.g., decreases in invertebrate abundances, decreases in amphibian populations, increases in fine sediments, etc.). We will utilize both concepts of weight of evidence.

Behind the weight of evidence approach is a belief that much of the information and insight about temporal change will be contained in species presence, absence, and abundance. Multivariate analyses can be used to efficiently explore the data and identify progressive changes (Figure 1). Two specific techniques for analyzing plot data include assessing cumulative plot dissimilarity over time (Phillipi et al. 1998) and outlier determination and control chart development (McBean and Rovers 1998, Anderson and Thompson 2004) (Figure 2). Compositional changes can provide compelling evidence that a meaningful ecological event has occurred or that an ecological threshold has been exceeded (Clarke and Warwick 2001, Anderson and Thompson 2004). At a minimum, cumulative dissimilarity ordinations (Figures 1-2) will be developed for each sampling frame from each park for the first 15 years of the program.

Philippi et al. (1998) suggest tests for trends in matrices of similarity indices: (1) Non-parametric multivariate analysis of variance can be used with a matrix of dissimilarities which can be partitioned into residual sums of squares to test for trend from the baseline condition (time 1, or another time period or reference). Significance is determined through a test using randomization of date labels. (2) Mantel test of a locational dissimilarity matrix to the temporal time difference matrix. Randomization following the traditional Mantel test then tests for significance of association between time and species composition (Manly 1997).

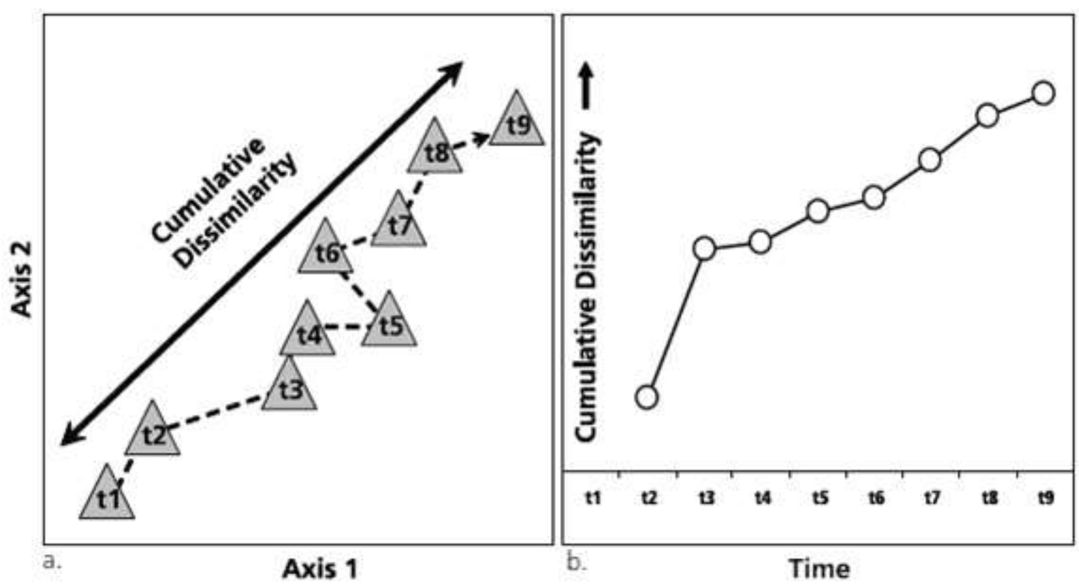

Figure 2. Cumulative change in species composition over nine sampling seasons. a.) An idealized two-dimensional ordination diagram illustrating the compositional position of a site at time one through nine, where Euclidean distance between each year (i.e., time steps t1, t2...t9) is proportional to species dissimilarity. The solid two-headed arrow is an ordination that illustrates the cumulative dissimilarity (progressive compositional change) over the whole period. b.) A graph of cumulative dissimilarity between the first year sample and successive years (i.e., t1 to tn). Note that the change is positive and sustained, suggesting a clear trend of changing composition over time.

SOP #22: Data Analysis and Reporting (continued).

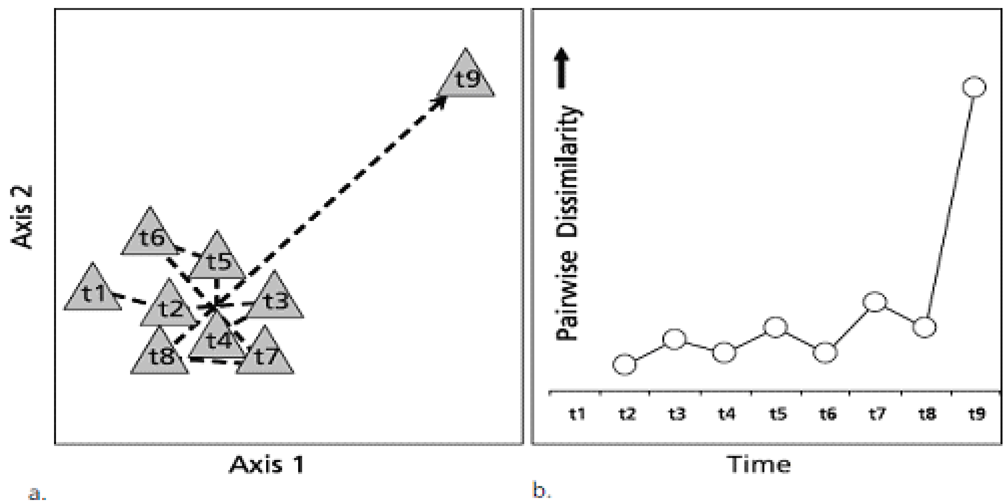

Figure 3 Year-to-year change in species composition over nine sampling seasons, with a major change at year nine. a.) An idealized two-dimensional ordination diagram illustrating the compositional position of a site at time one through nine where Euclidean distances between each pair of years (i.e., time steps t1, t2...t9) are proportional to pairwise species dissimilarity. The dashed arrow follows the year-to-year change in composition. b.) A graph of pairwise dissimilarity between each pair of successive time steps from years one to nine. Note that the composition is similar, but slightly variable in years one to eight, with a major change in year nine.

Other tests for progressive trend in assemblage data exist, such as the canonical analysis of principal coordinates (CAP) as proposed by Anderson and Willis (2003) and Anderson and Robinson (2003), and the perMANOVA test. The CAP analysis can be implemented in the R software vegan package with the capscale() function. Also, perMANOVA could be used to test for differences amongst sampling periods, amongst sites, and the error term would be the site by sampling period interaction (Anderson 2001). This can be implemented in the vegan package as well with the adonis() function; this is another permutation approach so computational time is high and the number of iterations used may have to be adjusted.

This report will also explore the standardization of the trends analyses, allowing future Analysis and Synthesis reports to include repeatable trend analyses through preparation of standardized ”R” scripts, and other analyses incorporating new annual data. It is recognized that the current software of choice is “R” but that future analyses may use a yet undeveloped software package.

We also expect that new techniques will emerge for studying trends that allow complex dynamics of species composition changes to be more clearly demonstrated. Emerging techniques will also be considered, and if applicable, applied to the trends Analysis and Synthesis report.

The essential “statistical toolboxes” for these analyses are listed in Table 2. Time series analysis (i.e., trends) is a topic spanning several textbooks filled with multiple techniques and approaches, and even an elementary introduction is beyond the scope of this SOP. However, a good starting point for these analyses will be two of the most elementary forms of time series, and these should be the backbone of the trends reports. To assist in the implementation, some guidelines are presented below.

SOP #22: Data Analysis and Reporting (continued).

Table 2. Proposed analyses for trend detection in Analysis and Synthesis Report 5. DOC = Dissolved Organic Carbon; ANC - Acid Neutralizing Capacity; IBI = Index of Biological Integrity, respectively. * = note that although these parameters are "univariate," they are derived from a broader suite of multivariate information, and being tested with univariate techniques, provide a robust assessment of trend.

Univariate parameters	Analytical tests	References	Proposed Software
Stream Physical Parameters -			
e.g., pool/riffle ratios, sinuosity, fish cover, etc.			
Chemistry -			
Anions, cations, DOC, nutrients, ANC	Parametric and non-parametric time series analysis (Regression models and Mann-Kendall rank correlation tests).	Quinn and Keough (2002), Chatfield (2006), Zar (2009)	Systat, "R", or similar
Biological* -			
Taxa richness, Shannon Index, Hilsenhoff Biotic Index, O/E scores, IBI, Fish condition index, Chlorophyll biomass			
Multivariate			
Macroinvertebrate assemblages	Indices of multi-variate seriation	Warwick and Clarke (1991), Philippi et al. (1998), Clarke and Warwick (2001)	Primer-E, PC-ORD, or similar

SOP #22: Data Analysis and Reporting (continued).

Linear Regression – Although multiple models of linear regression exist, reporting and interpretation of trend will be based on (1) slope estimate and standard error of slope; and (2) significance of slope via analysis of variance (ANOVA) F tests. The slope estimate provides the effect size of the trend (if any) and the direction, positive or negative. The standard error of the slope is an estimate of the precision of the slope. The actual effect size of the slope should be evaluated by the Project Lead for biological significance. The statistical significance is provided by the ANOVA F test (Quinn and Keough 2002). In most circumstances, the Klamath Network will use an α level (Type I error; chance of falsely rejecting a null hypothesis) of 0.10. A higher than usual α level (usually set at 0.05) is justified due to the NPS concern of false negatives (saying that no change has occurred when an impact, has in fact, happened [Irwin 2008]).

Mann-Kendall Trends Analysis – This is a non-parametric test for trends based on the Kendall's Tau (τ), a rank-order correlation coefficient of concordance. For example, if in five time periods (1 – 5), the response value increases with each period, there will be 100% concordance. If only four of the five are in concordance, there would be closer to 80% concordance.

Indices of Multivariate Seriation – This is a multivariate correlational test similar to the Mann-Kendall Trends Analyses. However, the correlation is tested between the elements of two symmetrical matrices: one based on the ecological similarity (measured with a similarity index, such as Bray-Curtis from assemblage data) and one based on temporal distances between samples (Clarke et al. 1993). A correlation coefficient is calculated by ranking the order of the elements and calculating the Kendall's Tau for concordance. Similar to the Mann-Kendall test, significance is tested by randomizing one matrix element and comparing the observed correlation coefficient to the resulting randomized distribution (Clarke and Warwick 2001). Indices of multivariate seriation can be used to assess trends in multivariate assemblages for both park-wide assessments and single revisited sites.

Resource Briefs
Upon completion of each major report, a one or two page resource brief will be prepared for dissemination to resource managers and the public. Resource briefs are intended to convey recent activities and key finding and to provide a succinct introduction to the topics in the more substantive Annual and Analysis and Synthesis reports and the Klamath Network Internet web site. Generally, the audience of the resource brief will be park resource staff, and secondarily, interpreters. Format will follow the standard resource brief template in use by the Network at the time.

Guidelines for Standardized Metrics for Water Quality and Aquatic Community Analyses
The purpose of this section is to ensure standardization so that analyses of data from this program are comparable across years.

pH: Because pH is a logarithmic value, pH must be converted to the antilog (i.e., raw hydrogen ion concentration), averaged, then reconverted to pH. This should be done for averaging the cross-sections at each stream site (see example in Table 3). However, when averaging pH among streams (for example to calculate an average pH for all streams of Lassen Volcanic National Park), a standard average should be used.

Table 3. Example depth and pH readings taken in a hypothetical lake and how to average pH readings. Note that a straight average of the pH readings = 7.358; not 7.348, the correct value.

Cross-section	pH	Raw value[1]	Average raw value	Average pH[2]
1	7.3	5.01187E-08		
2	7.25	5.62341E-08		
3	7.4	3.98107E-08		
4	7.4	3.98107E-08	4.4822E-08	7.348
5	7.3	5.01187E-08		
6	7.3	5.01187E-08		
7	7.56	2.75423E-08		

[1]can be calculated in MS Excel using "=POWER(10, -value)", where 10 is the logbase, and "value" is the measured pH; note that it must be negative. [2]Average value reconverted using the "=-LOG(value,10)" function in MS Excel where value is the averaged raw value and 10 is the baselog.

Taxonomic Resolution/Operational Taxonomic Units

Taxonomic resolution may vary from site to site and year to year. One reason is that mature invertebrates (i.e., later instars of insect larvae) are more likely to have developed the diagnostic features necessary for identification. Another reason is that some taxa have only genus level keys (e.g., Ephemeroptera) and others have better developed species keys (e.g., Coleoptera: Dytiscidae). Damaged individuals may also limit taxonomic resolution. Lastly, taxonomic expertise of the individual identifying the specimen may cause differences in resolution.

Standardization of taxonomic resolution is accomplished by requiring contract laboratories to only employ taxonomists certified by the North American Benthological Society (www.benthos.org) and by timing the collection of samples to similar times of the year. However, the varying amounts of taxonomic resolution present a problem in determining the total number of unique taxa in which to base taxa richness and Shannon index calculations. To this end, the contract laboratory provides the determination of which taxa not identified to the lowest practical level are “unique.” This allows the taxonomist to identify a species to genus/species level for one specimen, and only identify a specimen of the same family to the family level. If he or she determines that the specimen keyed to family level is “unique,” this indicates that the specimen is probably not represented by the individuals identified to the genus/species level and should be treated as a separate new taxon, despite the reduced resolution.

In general, the standard for the Klamath Network is to report at the raw taxonomic level, so that all taxonomy is presented, regardless of the taxonomic resolution.

In certain circumstances, the Project Lead may also convert the raw taxa to “Operational Taxonomic Units.” Operational Taxonomic Units (OTUs) is a process where the investigator combines taxa into a coarser level across samples to facilitate analyses at a common level of taxonomy. A single class of taxa combined in such a way is an “OTU.” This reducing step of using coarser taxonomy must *be well documented and reported*, and the raw data must always be retained. The process of creating OTUs should be conducted across the entire set of samples being analyzed; it cannot just be done to a subset. For long-term monitoring, there is also a risk that a previous investigator may assign OTUs at a certain coarse level and a later investigator may choose a different level. Hence, whenever an OTU is used for analyses, the current investigator must apply it to the entire dataset of raw taxonomy *and not just the current subset.*

Bioassessment Tools
We use two general classes of bioassessment tools in our protocol (1) Multi-metric models (IBIs, MMIs) and (2) Multivariate predictive models (O/E scores). Both of these tools can be distilled into clear, readily interpretable condition assessment for managers.

Although these two approaches vary in their methodology, we maintain that an integrated approach to bioassessment using both approaches gives a better assessment than any single tool by itself. For example, the North Coast IBI of California has overrated some sites in comparisons to O/E scores (Rehn et al. 2005). The O/E scores are more sensitive to loss of specific taxa, whereas the IBI is sensitive to changes in assemblage structure and function. Although we expect both methods to give comparable results in most circumstances, the deviations from this are not a paradox, but rather an indicator that suggests a closer inspection of the site. These deviations may also signal specific stressors (Rehn et al. 2005).

Likewise, multiple models of IBIs or O/E scores exist. In this protocol, we utilize both locally specific IBIs (Northern coastal California, and Western Oregon Mountains) and regionally broader IBIs (EPA EMAP models). Regionally-specific models have the advantage of being calibrated to be both precise and accurate. Broader models, fitted over a wider range of environmental conditions, are considered less precise. However, recent work has shown broad corroboration between local and regional models (Meador et al. 2008). The use of a region-wide model has the benefit of being able to apply a single metric to a network-wide assessment.

In addition to IBIs and O/E scores, we use three other standard reporting metrics: Hilsenhoff Biotic Index (HBI); Shannon Index; and relative abundances. Rationale and interpretation for these metrics are included in their description.

Indices of Biotic Integrity
Indices of Biotic Integrity (IBI) is a bioassessment tool pioneered by James Karr in assessing the health of fish communities (Karr 1981). Since then, it has been applied to and become the traditional approach to analyzing macroinvertebrate assemblage data (Stoddard et al. 2005). Often called B-IBI (Benthic – Integrity of Biological Integrity), various composition, tolerance, and richness characteristics are summarized for a sample set, resulting in a set of candidate metrics. A subset of 5 to 10 “best performing” (based on their quantitative ability to distinguish disturbance) metrics are then combined into a multi-metric model and scored on the basis of regional reference sites. More information on IBIs can be found in Karr and Chu (1999).

Metrics selected for inclusion often include life history or habit variables of taxa (e.g., the number of “clingers,” invertebrates who cling to the surface of rocks in swift flowing water). The standard source for use in the Klamath Network for life history, feeding groups, or habits will be the EPA list of variables (Barbour et al. 1999), provided in Appendix P.

Although most prominent for use with invertebrates, IBIs are also used for stream vertebrate assemblages (Stoddard et al. 2005), even though they are more common in the streams and lakes of the midwestern and eastern US, which are characterized by a more diverse assemblage of fish. Some regional models (e.g., Mebane et al. 2003) are developed for fourth order streams or larger and are sampled with boat electrofishing. However, IBIs developed for wadeable streams using single pass electrofishing do exist.

SOP #22: Data Analysis and Reporting (continued).

Table 4 shows the available regional and west-wide IBIs (vertebrate and invertebrate) applicable to the parks of the Network, the four available models (three invertebrate and one vertebrate) are described in detail below.

Table 4. Park units and available IBI models applicable to the units.

Park Unit	EPA West-wide	CA Northern Coastal Region	OR Western streams	EPA West-wide Vertebrate
CRLA	X		X	X
LAVO	X			X
ORCA	X	X	X	X
REDW	X	X		X
WHIS	X	X		X

California Northern Coastal Region B-IBI

The California Regional Water Quality Control Board, North Coast Region, in conjunction with the California Surface Water Ambient Monitoring Program (SWAMP), has developed a series of B-IBIs for the coastal region and for the Klamath Mountains of northern California (Rehn et al. 2005, included in Appendix P). The coastal B-IBI can be used for stream monitoring in REDW, whereas the Klamath Mountain B-IBI can be used for both WHIS and ORCA. It should be noted that ORCA was not in the geographic region that the metrics were developed for; however, because ORCA is in the Klamath Mountain Omernik level III ecoregion, use of the Klamath B-IBI should provide useful results.

To calculate the B-IBI for these regions:

1. Obtain or calculate the following eight component metrics from the macroinvertebrate dataset for each stream reach sampled:
    a. Ephemeroptera, Plecoptera, and Trichoptera Richness
    b. Coleoptera Richness
    c. Diptera Richness
    d. % Intolerant Individuals
    e. % Non-Gastropoda Scraper Individuals (adjusted)
        i. Predict metric at each site based on watershed area (in km^2):
        $y = -0.089(\log_{10} Watershed\ area) + 0.433$
        ii. Calculate the difference between the observed value and predicted value (the residual).
        iii. Add 0.296 (a constant) to the residual and multiply by 100 to convert to percent.
    f. % Predator Individuals
    g. % Shredder Taxa
    h. % Non-Insect Taxa
2. Score each component metric based on the following table:

SOP #22: Data Analysis and Reporting (continued).

Component Metric	Park Unit	Assigned Score										
		0	1	2	3	4	5	6	7	8	9	10
EPT Richness	(All)	0-2	3-5	6-7	8-10	11-12	13-15	16-17	18-20	21-22	23-25	>25
Coleoptera Richness	(All)	0	1		2		3		4		5	≥6
Diptera Richness	(All)	0	1	2	3	4	5	6	7	8	9	≥10
% Intolerant Individuals	(All)	≤ -5	-4-0	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	≥41
% Non-Gastropoda Scraper Individuals	(REDW)	0-4	5-8	9-12	13-16	17-20	21-24	25-28	29-32	33-36	37-40	≥41
	(ORCA, WHIS)	0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17	≥18
% Predator Individuals	(REDW)	0-1	2	3-4	5	6-7	8	9-10	11	12-13	14-15	≥16
	(ORCA, WHIS)	0-1	2-3	4-5	6-7	8-9	10-12	13-14	15-16	17-18	19-21	≥22
% Shredder Taxa	(REDW)	0-1	2-3	4-5	6-7	8-9	10-11	12-13	14-15	16-17	18-19	≥20
	(ORCA, WHIS)	0-1	2	3-4	5	6-7	8	9-10	11	12-13	14-15	≥16
% Non-Insect Taxa	(All)	≥57	52-56	47-51	41-46	36-40	30-35	25-29	19-24	14-18	8-13	0-7

3. Sum the metrics from all eight component metrics and multiply by 1.25 for a final score on a 100 point scale.
4. An example using two streams from the pilot project: Godwood Creek and Redwood Creek (both in REDW):

Component Metric	Godwood Creek		Redwood Creek	
	Value	Score	Value	Score
EPT Richness	38	10	23	9
Coleoptera Richness	8	10	5	9
Diptera Richness	15	10	18	10
% Intolerant Individuals*	15	4	31	8
% Non-Gastropoda Scraper Individuals	26	6	12	2
% Predator Individuals	13	8	23	10
% Shredder Taxa	16	8	4	2
% Non-Insect Taxa	15	8	9	9
	Sum of scores:	64		59
	Final Score (Adjusted by 1.25):	80		73.75

5. Stream conditions can now be assigned based upon to their scores:
   a. 0-20: Very Poor
   b. 21-40: Poor
   c. 41-60: Fair
   d. 61-80: Good
   e. 81-100: Very Good
6. Furthermore, allocation of “impaired” or “unimpaired” condition can be assigned to each stream reach at the threshold value of 52 (two standard deviations below the mean reference [Rehn et al. 2005]). Streams with B-IBI scores above 52 are "unimpaired" and

streams 52 or below are rated “impaired.” Proportion of streams and stream miles impaired in each park can now be assigned.

Western Oregon IBI

The state of Oregon developed an IBI for western Oregon streams (OWEB 1999; original methods on IBI calculation are included in Appendix P). In general, scores are calculated as above, with slight modifications: (1) there are 10 metrics total; (2) scores for each metric vary from 1 to 5; and (3) the final score is out of a total of 50. Metrics and scoring criteria are provided below:

	Score		
Component Metric	5	3	1
Taxa Richness	>35	19-35	<19
Ephemeroptera Richness	>8	4-8	<4
Plecoptera Richness	>5	3-5	<3
Caddisfly Richness	>8	4-8	<2
Sensitive Taxa	>4	2-4	<2
Sediment Sensitive Taxa	>2	1	0
Modified HBI	<4.0	4-5	>5.0
% Tolerant Taxa	<15	15-45	>45
% Sediment Tolerant Taxa	<10	10-25	>25
% Dominance of the most common taxa	<20	20-40	>40

Stream condition is then based on the score:

- >39: No impairment
- 30-39: Slight impairment
- 20-29: Moderate impairment
- <20: Severe impairment

Lassen Volcanic National Park IBI – the Missing IBI, and Broad Scope EPA MMIs

Lassen Volcanic National Park poses an interesting conundrum in its geographic location. The state of California places LAVO in the central valley region by the State Water Resources Control Board (SWRCB). Indices of Biotic Integrity have been developed for the Central Valley by the SWRCB, but they focus on lower elevation streams not represented in LAVO. The streams of LAVO are more akin to the streams of the Sierra Nevada. Hence, as of this writing, there is no regionally applicable IBI model to use for LAVO. The Project Lead should periodically consult with the state of California Water Resources Board to stay informed of the possible development of an applicable model.

However, the EPA EMAP project has developed a west-wide model of an IBI, called a Multi-Metric Index (MMI) (the two terms are synonymous [Stoddard et al. 2005]). As an IBI covering an entire western region, more variance is possible; it has not been regionally calibrated. Until an available regional model is developed for the LAVO region, this should be utilized. As an available model, however, it has the advantage of being applicable to all parks of the Klamath Network. This makes comparability possible for a Network-wide assessment.

SOP #22: Data Analysis and Reporting (continued).

The EMAP west-wide (mountains) MMI/IBI is calculated as the following (and relevant portions included in Appendix P):

1. For each sample, a random draw of 300 individuals is taken (without replacement). This can be accomplished using a built-in function on the KLMN Database or another software program such as R.
2. Component metrics for each sample are calculated:

Metric	Ceiling	Floor
Burrower % Individuals	0	20
EPT Distinct Taxa Richness	28	5
Non-Insect % Individuals	0	65
Omnivore % Distinct Taxa	0	6
Percent of Individuals in Top 5 Taxa	32	90
Tolerant % Distinct Taxa	0	30

3. Metrics are scored on a scale from 0-10:
    a. Metrics below or equal to the “floor” of 5% are given “0.”
    b. Metrics above or equal to the “ceiling” of 95% are given “10.”
    c. Metrics in between the floor and ceiling are linearly interpolated:
        i. Scores can be interpolated with the following formula:
        $$Score = \frac{(measured\ value - floor)(10)}{ceiling - floor}$$
        ii. For example, if the dominant five taxa of a sample = 61%, the score is calculated as: $Score = \frac{(61-32)(10)}{90-32}$ = 5
4. The six component metrics are then summed and multiplied by 1.666 to create a 0 to 100 scale.
5. Although the MMI gives a score that could be graded on the California scale (above), the EPA rating system uses regional “least-disturbed reference sites” to base grading. Sites that are worse than 75% of the value of these least-disturbed sites are ranked “fair” and sites that are worse than 95% of the value are ranked “poor.” If the California grading system is used, reporting should always include a relevant disclaimer.

EPA EMAP Aquatic Vertebrate MMI

The EPA EMAP MMI (or fish/amphibian IBI) is calculated as:

1. For each sample site, calculate the following component metrics (using taxa information included in Appendix P):
    a. Percent of individuals that are rheophilic (fast-water) sensitive.
    b. Percent of taxa that are supertolerant.
    c. Percent of individuals that are native, sensitive, invertivore/piscivore.
    d. Percent of taxa that are lithophile (gravel spawning fish).
    e. Percent on individuals that are in the family Cyprinidae.
    f. Percent of taxa that are native, non-tolerant, and long-lived.
    g. Percent of individuals that are alien (non-native).
2. Express percent as proportion (e.g., 0.5 instead of 50%).
3. Score on scale of 0 – 10 as above for EPA EMAP invertebrate MMI:

SOP #22: Data Analysis and Reporting (continued).

Component Metric	Ceiling	Floor
% individuals Rheophilic sensitive	1	0
% taxa supertolerant	0	0.2
% individuals native, sensitive, invertivore/piscivore	1	0
% taxa lithophil	1	0.3
% individuals Cyprinidae	0	0.5
% taxa native, non-tolerant, long-lived	1	0
% individuals alien	0	1

4. Use the same formula for linearly interpolating score values as above.
5. Sum component metrics; multiply result by 1.42 to put on a 0 – 100 scale.
6. The EPA does not give guidelines on interpretation of scores, but the 0-20: Very Poor; 21-40: Poor; 41-60: Fair; 61-80: Good; and 81-100: Very Good are a useable scale, with the qualification that it was adapted from the SWAMP macroinvertebrate IBI scoring.

Observed/Expected Ratios

A fundamental estimate of impairment is the impoverishment of a biological community. Observed/expected ratios, also known as "taxa lost" or RIVPACS (River InVertebrate Prediction and Classification System) models, provides a conceptually simple method of bioassessment where the number of collected taxa is compared to the number expected under unimpaired conditions (Hawkins et al. 2000). Values below 1.0 represent "lost" taxa, indicating impairment. The ratio O/E is hence readily interpretable to managers and the public. For example, an O/E score of 0.8 indicates that only 80% of the expected taxa were present.

Although conceptually simple, the derivation of the expected values require a high level of statistical knowledge and a large sample size comprised of both reference sites and impacted sites. O/E values, however, are the primary method of the EPA to assign water quality grades based on macroinvertebrate assemblages (US EPA 2006), and are a valuable tool to monitoring the wadeable streams of the Klamath Network.

Calculation of O/E scores for the monitoring efforts of the Klamath Network can be obtained in one of two ways: (1) employ the National Aquatic Monitoring Center as the contract laboratory, which can provide O/E values based on EPA models, or (2) utilize the online software of the Western Center for Monitoring & Assessment of Freshwater Ecosystems (http://cnr.usu.edu/wmc/), which has complete instructions on how to use both state-specific and west-wide models to generate O/E scores. The web site is currently being upgraded, so specific procedures cannot be detailed at this time. The final product of this draft protocol will include specific instructions as the online software is updated.

Hilsenhoff Biotic Index (HBI)

This index is specific to macroinvertebrates. It is a weighted average of tolerance values derived from empirical observations of macroinvertebrate responses to pollution (Hilsenhoff 1987, 1988). It is calculated as:

$$HBI = \frac{\sum n_i \, a_i}{N}$$

SOP #22: Data Analysis and Reporting (continued).

Where n_i = the number of individuals for taxa i, a_i = the assigned tolerance value of taxa i, and N = the total number of individuals for a sample.

The Hilsenhoff Biotic Index (HBI) summarizes the overall tolerances of the taxa collected; changes in a site's HBI can be viewed as an indication of community change (Hilsenhoff 1987, 1988). This index is used to detect nutrient enrichment, high sediment loads, low dissolved oxygen, and thermal impacts. Taxa are assigned an index value from 0 (taxa normally found only in high quality unpolluted water) to 10 (taxa found only in severely polluted waters). Shifts in HBI from low values to high values indicate a shift toward a more pollution tolerant assemblage; a shift from high values to low values indicates a change towards a more intolerant assemblage.

For consistency, a single source for tolerance values should be utilized. The source for this protocol is tolerance values developed by Mr. Robert Wisseman of Aquatic Biology Associates and is available at: http://www.cbr.washington.edu/salmonweb/taxon/. This source has been chosen because: 1) it was developed specifically for Pacific Northwest taxa, and 2) it includes non-insect tolerance values.

One advantage of the HBI is that tolerance values have been developed for Order, Family, and Genus/species. Hence, individuals that were only identified to Family can still be incorporated in the index without making assumptions or collapsing taxonomic information.

Species Abundances
Relative abundances should be calculated for a) Macroinvertebrates (per square meter) b) Fish (catch per unit effort), and c) Amphibians (catch per unit effort).

The abundance, density, or number of aquatic organisms per unit area or catch per unit effort is an indicator of habitat availability and fish food abundance. Abundance may be reduced or increased depending on impacts or pollutants. Increased organic enrichment typically causes large increases in abundance of pollution tolerant taxa. High flows, increases in fine sediment, or the presence of toxic substances normally decrease invertebrate abundance.

Macroinvertebrates, for logistical reasons, are sub-sampled during processing. Although the sub-sampling of macroinvertebrates is quantitative in nature, additional potentially compounding error is added to the sample. Hence, data interpretation and reporting macroinvertebrates should focus on relative abundances. Although abundances for individual taxa can be ecologically relevant, the presentation of abundances for 100+ taxa over a long-term time series does not lend itself to easily interpretable summaries. Hence, presentation of abundance data should be at the gross level for these groups (e.g., all macroinvertebrates per square meter). Abundance of individual taxa should only be included if there are special considerations justifying it (e.g., endangered or invasive species).

Shannon Index (H')
Ecological diversity is a measure of community structure defined by the relationship between the number of distinct taxa and their relative abundances, incorporating both into a single measure. A Shannon Index measurement of 0 indicates that the assemblage is composed of only one species, and increases upward with increasing species richness and eveness. Typical values range from 0 to 3.

SOP #22: Data Analysis and Reporting (continued).

This information index incorporates both relative abundance and taxa richness (Magurran 2004). It is calculated as:

$$H' = -\sum p_i \ln p_i$$

Where p_i = the proportion of the *i*th species (e.g., abundance of taxa *i* divided by the total abundance of the sample).

The calculation is straightforward and easily done in MS Excel or another spreadsheet. However, two important considerations must be made: 1) taxonomic resolution and 2) which logarithmic base to use. Taxonomy should be based on unique taxa (see above). Although examples of using different logarithmic base for the transformation exist in the literature, there is growing momentum to standardize on the natural log (ln) (Magurran 2004). **All Shannon Indices calculated for this monitoring program should use the natural log.**

Water Quality Exceedances

Although this protocol is not designed to monitor for water quality standards exceedances, where measured values exceed standards, reporting should include any instances or indications of exceedances where encountered. Because the protocol sampling is a single point in time, any reports of exceedances should not constitute a call for management action. Instead, it is a signal that there may be impairment and the parameter exceeded should be investigated using state standards (e.g., 4 day average of parameter X) to determine actual exceedance. If there are indications of exceedances, follow up monitoring by the park units with assistance from the networks should be implemented.

Both the state of California and the state of Oregon have promulgated water quality standards. However, many of the standards are for toxic substances (e.g., Polyaromatic Hydrocarbons) and do not overlap with monitored parameters under these protocols. Of the California standards, they have yet to develop standards for the monitored parameters. For Oregon, most standards are centered on allowable increases or decreases from natural conditions. Table 5 presents the Oregon standards, along with National Park Service and Environmental Protection Agency standards.

These standards may be updated, expanded, and revised by the respective agencies. The Project Lead should periodically (once per sampling event) check for updates. The sources used in Table 5 are:

- Oregon - http://www.deq.state.or.us/WQ/standards/standards.htm (accessed on 21st January 2009).
- California - http://www.waterboards.ca.gov/water_issues/programs/water_quality_goals/search.shtml (accessed on 16th April 2010).
- EPA Standards - http://www.epa.gov/waterscience/criteria/wqctable/ (accessed on 21st January 2009)
- NPS Standards – Embedded in NPS Storet, v. 1.71.

SOP #22: Data Analysis and Reporting (continued).

Table 5. Water quality thresholds for state of Oregon and California, EPA and NPS standards.

		Oregon	California	EPA Standards			
Parameter	NPS Standards	Department of Environmental Quality	Drinking Water	Drinking Water	Health Advisory	National Ambient Water Quality Criteria	Region 10 Collaborative Guidance
Alkalinity (mg/l)	> 25	> 20				> 20	
Chloride (mg/l)			< 250 mg/l			< 230[1,4]; <860[2,4]	
Dissolved Oxygen (mg/l)	> 4	> 8 mg/l (Cold Water Aquatic Life)				> 8.0 1 day minimum (water column)	
Total Nitrogen (as NO2 + NO3) (mg/l)			< 10	< 10			
pH	> 6.5	6.5 to 9 (Klamath basin); 6.5 to 8.5 (Rogue basin)		6.5 to 8.5		5 to 9[3], 6.5 to 9[1](max)	
Sodium (mg/l)					< 20		
Sulfate (mg/l)				< 500		< 250[3]	
Temperature, (fall, winter, spring) (7 day average of daily maximum)							< 9 °C (Bull trout); < 13 °C (general salmon and trout); < 14 °C (Steelhead)
Temperature, maximum (7 day average of daily maximum)		< 18 °C (Salmon and Trout Rearing and Migration (ORCA)); <12 °C (Bull trout spawning and juvenile rearing (CRLA))					< 12 °C (Bull trout); < 16 °C (salmon and trout core rearing); < 18 °C (salmon and trout noncore rearing); < 20 °C (salmon and trout migration)
Turbidity (NTU)	< 50	< 10% above natural	< 1	< 1			

[1]Standard for Freshwater Aquatic Life Protection (4 day average); [2]Maximum 1 hour concentration; [3]Taste and odor standard;[4]Chloride standards only apply when dominant cation is Sodium.

SOP #22: Data Analysis and Reporting (continued).

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